LITERATURE CITED

1. M. P. Gorizontova, O. V. Alekseev, and A. M. Chernukh, Byull. Éksp. Biol. Med., No. 3, 22 (1975).
2. M. P. Gorizontova, in: Problems in the General Theory of Disease [in Russian], Moscow (1976), pp. 80-83.
3. M. P. Gorizontova and A. M. Chernukh, Byull. Éksp. Biol. Med., No. 6, 645 (1976).
4. M. P. Gorizontova, O. A. Gomazkov, I. R. Ignat'eva, et al., Byull. Éksp. Biol. Med., No. 11, 515 (1981).
5. A. M. Chernukh, M. P. Gorizontova, and V. S. Shinkarenko, Byull. Éksp. Biol. Med., No. 10, 507 (1977).
6. O. Hägemark, T. Hökfelt, and B. Pernow, J. Invest. Derm., 71, 233 (1978).
7. K. Hecht, P. Oehme, I. A. Kolemetseva, et al., in: Neuropeptides and Neural Transmission, ed. C.A.A. Marsan et al., New York (1980), pp. 159-164.
8. I. A. Kiernan, Quart. J. Exp. Physiol., 60, 123 (1975).

RHEOLOGIC PROPERTIES OF PURIFIED HEMOGLOBIN SOLUTIONS MIXED WITH BLOOD

É. V. Shtykova, G. M. Morozova, E. P. Vyazova, and V. P. Matvienko

UDC 615.384:547.963.4].033

KEY WORDS: hemoglobin; viscosity; microcirculation; rheologic properties; yield point.

The development of blood substitute solutions able to perform a gas transporting function *in vivo* is an important development in modern transfusion science [2]. One possible method of solving this problem is by using a natural oxygen carrier, in the form of solutions of native hemoglobin or its modifications, as the transfusion medium [4, 7,8]. The blood substitute must not only perform certain physiological functions (hemodynamic, gas-transporting, etc.), but it must also be suitable from the point of view of its rheologic properties.

This paper gives the results of a study of some rheologic and viscosity parameters of concentrated hemoglobin solutions, free from stromal impurities and from procoagulant activity.

EXPERIMENTAL METHOD

Hemoglobin solutions obtained by the method described previously were used [3]. The relative viscosity of purified hemoglobin in physiological NaCl solutions was determined by means of "Ubbelohde" capillary viscometers. The rheologic characteristics were studied *in vitro* on a "Low Shear 30" rotation viscometer (from Contraves, Switzerland) at 37°C within a range of shear velocities of between 0.05 and 128.5 sec^{-1}.

To study the effect of solutions of extraerythrocytic hemoglobin on the suspension properties of blood comparative *in vitro* models were set up with replacement of 25, 50, and 75% of blood by 10%, 15%, and 20% solutions of purified hemoglobin and clinical preparations of dextran (6% polyglucin and 10% rheopolyglucin), and the rheologic properties of the resulting mixtures were investigated. The final concentrations of free hemoglobin in the blood plasma are given in Table 1.

EXPERIMENTAL RESULTS

Measurement of the relative viscosity of hemoglobin in concentrations of between 5 and 25% showed that the viscosity of even higher concentrated solutions (25%) is low, about 3.0

Laboratory of Blood Substitutes and Oxygen Carriers and Laboratory of Experimental Transfusion, Central Research Institute of Hematology and Blood Transfusion, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 9, pp. 19-21, September, 1982. Original article submitted March 15, 1982.

0007-4888/82/4909-1181$07.50

TABLE 1. Concentration of Free Hemoglobin in Plasma (in %) after Different Degrees of Blood Replacement by Purified Hemoglobin Solutions

Replacement, %	Solution of purified hemoglobin		
	10 %	15 %	20 %
75	8	13	17
50	6	10	13
25	4	7	8

TABLE 2. Yield Points of Blood (in $mPa^{-\frac{1}{2}}$) following Different Degrees of Replacement by Hemoglobin, Polyglucin, and Rheopolyglucin Solutions

Replacement, %	Hemoglobin solution			6% polyglucin	10% rheopolyglucin
	10 %	15 %	20 %		
25	0,94	1,11	1,43	1,34	1,47
50	0,32	0,42	0,99	0,57	0,66
75	0,13	0,16	0,43	0,31	0,32

at 37°C. Such a low relative viscosity can be regarded as a virtue from the rheologic point of view, and it also means that additives of protein or polysaccharide nature can be added to the hemoglobin solution in order to create a complex plasma expander on its basis.

Flow curves of 10%, 15%, and 20% hemoglobin solutions in 0.9% NaCl, unlike flow curves of whole donors' blood, are practically linear and newtonian in character (Fig. 1). The small deviation from a linear dependence observed at shear velocities under 1 sec^{-1} can be explained as a manifestation of unavoidable multiplet interactions between protein macromolecules in concentrations of over 7% [5].

The study of the effect of solutions of extraerythrocytic hemoglobin on the suspension properties of blood yielded three families of blood flow curves representing replacement by hemoglobin solutions of different concentrations. One of them, relating to the blood flow curves for replacement by 20% purified hemoglobin solution is shown in Fig. 2. The presence of extraerythrocytic hemoglobin in the plasma in a concentration of 17% clearly did not increase the viscosity of the blood, even at low shear velocities. This indicates the absence of any possible additional intercellular interaction in the blood under the influence of free hemoglobin. On the whole the blood viscosity is reduced, and this is an advantage from the point of view of the microcirculation. Similar results with a more marked hemodilution effect were obtained after replacement of blood by 15% and 10% hemoglobin solutions.

Flow curves of whole donors' blood and flow curves for 50% replacement of blood by 10%, 15%, and 20% hemoglobin solutions and by preparations of polyglucin and rheopolyglucin are given in Fig. 3. Comparison of the curves shows that flow curves of mixtures of blood with hemoglobin solutions of different concentrations lie below the flow curve for whole donors' blood and for mixtures of blood with polyglucin and rheopolyglucin, and they thus have a lower viscosity, including at low shear velocities. Similar results were obtained after 25% and 75% replacement of blood by the above-mentioned solutions. It must also be pointed out that the viscosity of blood with rheopolyglucin rises more slowly with a fall in shear velocity than the viscosity of blood with polyglucin, which means that the use of rheopolyglucin is indicated in microcirculatory disturbances.

An important characteristic of blood as a Casson (structured) fluid is its yield point, i.e., the critical tension at which the three-dimensional structure of a stationary suspension begins to break up. The yield point is determined by extrapolation of the flow curve between Casson coordinates to zero shear velocity:

$$\sqrt{\tau} = \sqrt{\eta \frac{du}{dx}} + \sqrt{\tau_0},$$

where τ is the shear stress.

The results of calculation of the yield point for different degrees of replacement of blood by hemoglobin, polyglucin, and rheopolyglucin solutions are given in Table 2. The yield point of whole donors' blood, which we measured, averaged 2.2 $mPa^{-1/2}$. The data given show that addition of highly purified hemoglobin solutions to blood reduces its yield point by several times. It will be clear from Table 2 that only a 20% solution of hemoglobin is comparable in yield point with the widely used clinical preparations of dextran, namely polyglucin and rheopolyglucin. A more marked hemodilution effect is given by 10% and 15% hemoglobin solutions, and this is particularly important from the point of view of improving the microcirculation, while hemoglobin solutions also perform a gas-transporting function.

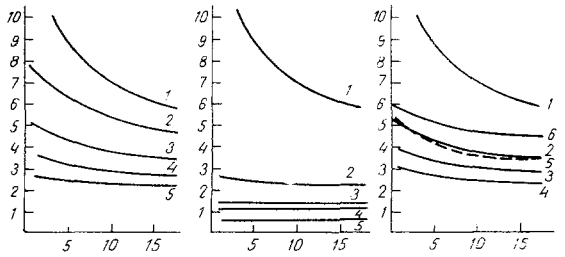

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Apparent viscosity as a function of shear velocity for whole donors' blood (1), and for 20% (2), 15% (3), and 10% (4) hemoglobin solutions and for physiological NaCl solution (5). Here and in Figs. 2 and 3: abscissa, shear velocity (in sec^{-1}); ordinate, apparent viscosity (in mPa•sec).

Fig. 2. Apparent viscosity as a function of shear velocity for whole donors' blood (1) and for 25% (2), 50% (3), and 75% (4) replacement of blood by 20% hemoglobin solution (5).

Fig. 3. Apparent viscosity as a function of shear velocity for whole donors' blood (1) and for 50% replacement of blood by 20% (2), 15% (3), and 10% (4) hemoglobin, polyglucin (5), and rheopolyglucin (6).

An important property determining the scope for the use of hemoglobin solutions as plasma expanders is their effect on the suspension stability of blood. A study of the erythrocyte sedimentation rate (ERS) showed that with an increase in the concentration of purified hemoglobin dissolved in plasma to 2% there was virtually no change in ESR; a further increase in the hemoglobin concentration caused a decrease in the ESR. For instance, when the hemoglobin concentration in plasma was 10 and 14%, ESR was reduced to 50 ± 5 and 35 ± 4% respectively of its initial value. Consequently, purified hemoglobin solutions, when mixed with blood, do not induce erythrocyte aggregation, whereas if erythrocytes are suspended in solutions of certain hemocorrectors and, in particular, of polyglucin the aggregation effect observed is accompanied by an increase in ESR [1, 6].

Hemoglobin solutions, freed from stomal impurities, thus have a low viscosity under all the flow conditions tested. Dilution of blood with hemoglobin solutions does not disturb its suspension stability and it significantly lowers the viscosity and yield point of the blood. All these findings indicate that effective blood substitutes, suitable for use in disturbances of the systemic hemodynamics, the microcirculation, and oxygenation of the body, can be created on the basis of hemoglobin solutions.

LITERATURE CITED

1. E. P. Mikhnovich, Probl. Gematol., No. 2, 53 (1976).
2. G. Ya. Rozenberg and A. A. Khachatur'yan, in: Oxygen Carriers [in Russian], Moscow (1979), p. 5.
3. G. Ya. Rozenberg, A. A. Khachatur'yan, E. P. Vyazova, et al., in: Proceedings of the First Soviet-American Symposium on Problem No. 6: Transfusion of Blood and Its Components and Prevention of Hepatitis in Cardiovascular Surgery [in Russian], Moscow (1978), p. 247.
4. N. A. Fedorov, V. S. Yarochkin, V. B. Koziner, et al., Dokl. Akad. Nauk SSSR, 243, No. 5, 1324 (1978).
5. A. A. Khachatur'yan, E. P. Vyazova, É. V. Shtykova, et al., in: Parenteral Protein Feeding and New Blood Substitutes [in Russian], Moscow (1979), p. 86.
6. Y. Goto and O. Aochi, Nagoya Med. J., 18, 53 (1974).
7. I. C. Sztuka, F. Bleser, S. Gaillard, et al., Anesth. Anal. Reanim., 33, 231 (1976).
8. B. Teisseire, D. Loisance, C. Soulard, et al., Rev. Fr. Transfus. Immunohemat., 20, 585 (1977).